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# Effect of $CO_2$ Laser on Healing of Cultured Meniscus

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***Background and Objective:*** **A new method to improve cartilage repair is clinically important. The enhancement of meniscal healing by low power $CO_2$ laser was investigated in an organ culture system.**
***Study Design/Materials and Methods:*** **A longitudinal or a radial defect was made in the avascular zone of rabbit menisci. Irradiation by $CO_2$ laser with 1 W (energy density 50 J/cm$^2$) and 2 W (energy density 100 J/cm$^2$) was used.**
***Results:*** **Histologic and scanning electron microscopic evaluations revealed that both energy densities of laser irradiation and the type of and the site of meniscal defect can influence the course and the outcome of meniscal healing. A marked increase in fibrochondrocytic proliferation and regeneration of collagen fibers were demonstrated in the meniscal defects irradiated by 100 J of $CO_2$ laser energy.**
***Conclusions:*** **The healing of meniscal defects could be promoted by low power $CO_2$ laser irradiation. Lasers Surg. Med. 20:172–178, 1997.** 

**Key words: laser; meniscal healing; organ culture**

## INTRODUCTION

Total and partial meniscectomies were once accepted as the proper treatment of a torn meniscus [1]. Later results from long-term study of total menisectomy showed degenerative changes of the operated knee joint [2]. Biomechanical studies prove that meniscus plays an important role in load transfer across the knee [3]. Consequently, more conservative managements have been advocated in dealing with the torn menisci to maintain the proper function of the knee joint [4].

In 1985, Webber and coworkers [5] demonstrated that cultured rabbit meniscal fibrochondrocytes were capable of proliferating and synthesizing sulfated proteoglycans. Later, they also showed that meniscal fibrochondrocytes in an organ culture system migrate into the defect to start the process of wound repair. Recently, low energy lasers have been shown to enhance tissue repair or healing in various tissues, to modulate human immune system, and to stimulate injured sciatic nerves [6–10]. Some preliminary results [11,12] suggest that lasers may be valuable for treatment of an injured meniscus by stimulating fibrochondrocytes proliferation in the wounded areas. Other researchers have also used low power lasers for stimulation of chondrocyte proliferation in hyaline cartilage, but the results are controversial [13,14]. We therefore designed an experimental model to study further the effect of

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laser irradiation on meniscal repair and to investigate the enhancement of the healing of meniscal injury using various energy levels of $CO_2$ laser irradiation.

## MATERIALS AND METHODS

### Animals

Ten adult New Zealand white male rabbits, each weighing between 2.0 and 3.0 kg, were randomly divided into three groups; group I (n = 4), group II (n = 4), and group III (n = 2). Group I and group II were the experimental groups irradiated with different laser energy. Group III was used as a control.

### Organ Culture

Rabbits were sacrificed by an injection of absolute alcohol. The knee joints were exposed under aseptic conditions. The lateral and medial menisci were removed and placed into petri dishes containing sterile Hank's balanced salt solution (Sigma, St. Louis, MO). Each meniscus was carefully trimmed by sharp dissection, leaving only the avascular portion. Defects were made according to Sherk's model with modification [11]. A full-thickness defect of either single longitudinal cut (0.1 × 3 mm, 1 mm away from the meniscal rim) or radial type (0.5 × 1 × 1 mm) was made in the avascular area of each trimmed meniscus (Fig. 1). Each defected meniscus was then placed in a 25 × 25 mm petri dish (Nunc, Denmark) containing 4 ml of Dulbecco's Modified Eagles' Medium (DMEM, GIBCO, Irvine, CA) supplemented with 10% fetal bovine serum (FBS, KC Biological Co.) and 0.1% penicillin/streptomycin (10,000 I.U. and 10,000 μg/ml, respectively). Organ culture of menisci was performed according to the methods of Webber et al. [15]. Subsequent incubation of the defected menisci was maintained at 37°C with 5% $CO_2$. Menisci of group I and II were incubated at least 12 hr before laser irradiation. All menisci, either laser-irradiated or unlased, were routinely cultured for 8 weeks by replenishing the medium and replacing the petri dish to maintain an adequate level of nutrition and prevent any possible contamination. After culturing for 8 weeks, menisci from each group were further prepared for histologic evaluation.

### Laser Irradiation

The defected experimental menisci (groups I and II) were irradiated with low power, continuous wave $CO_2$ laser (NIIC 15 Surgical $CO_2$ Laser System, Tokyo, Japan). Laser parameters were chosen based on the preliminary results of our investigation [16]. The output of $CO_2$ laser wave was a collimated beam with an indicator accuracy of ± 0.1 W. The beam size was 8 mm for all experimental menisci (groups I and II). In group I, the defected menisci were irradiated with 1 W (power density 2 W/cm$^2$) for 25 seconds (energy density 50 J/cm$^2$). The menisci in group II were irradiated with 2 W for 25 seconds (power density 4 W/cm$^2$; energy density 100 J/cm$^2$). Group III was not lased to serve as control.

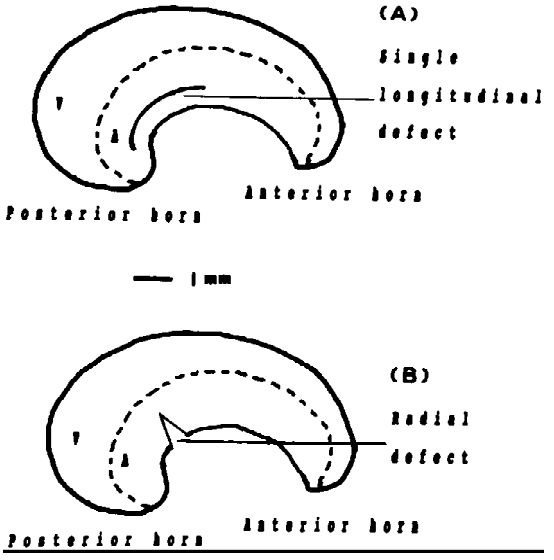



Fig. 1. A schematic drawing of full-thickness defects of either (**A**) longitudinal or (**B**) radial type, made near the concave edge of avascular zone of rabbit medial meniscus (V: vascular zone; A: avascular zone).

### Histologic Analysis

At the end of 8-weeks of culture, the defected menisci were processed for histologic examination on the effects of $CO_2$ laser on meniscal healing. Menisci were rinsed with normal saline and fixed in Bouin's solution (Sigma, St. Louis, MO), followed by dehydration in a serial concentration of alcohol from 70–100%, and decalcification in 25% formic acid. The fixed samples were immersed in xylene and embedded with paraffin. Cross sections of 5–6 μm in thickness were sliced from meniscal defects, stained with hematoxylin and eosin, and observed under light microscopy.

To prepare specimens for observation with scanning electron microscopy (SEM), samples were fixed according to the methods described above for light microscopy. The samples were fur-

ther dried at 30°C for 30 minutes and coated with gold under an ion coater (IT-2, Eiko Engineering Co., Tokyo, Japan). Subsequently, the samples were observed under a SEM (ABT, SX-30E, Tokyo, Japan).

## RESULTS

### Gross Observation

All the organ cultures were successfully maintained for 8 weeks. At week 8, the longitudinal meniscal defects in the control group remained unchanged, whereas those in groups I and II healed completely and could not be identified. Although the radial meniscal defects were still visible in all groups, a moderate decrease in the size of the meniscal defect was observed in groups I and II compared to control.

### Histologic Findings

After 8 weeks of incubation, menisci from each group were processed for histologic evaluation and observed under light microscope (LM). The longitudinal meniscal defect was almost closed in group II (Fig. 2), became a narrow gap in group I (Fig. 3), and remained unchanged in group III (Fig. 4). A significant number of fibrochondrocytes appeared in the vicinity of meniscal defects and fibrocartilage regeneration from both sides of the longitudinal meniscal defects were observed in both lased groups I and II. No obvious evidence of fibrocartilage regeneration along the longitudinal meniscal defect was observed in the unlased group III (Fig. 2). Fibrochondrocytic proliferation of the longitudinal meniscal defects in group I was comparatively less than that in group II.

A marked increase in fibrochondrocytic proliferation and matrix outgrowth was also observed along the radial meniscal defects in lased groups especially in group II (Fig. 5). No obvious matrix restoration and no significant multiplication of fibrochondrocytes were observed around the radial meniscal defects in the control group III (Fig. 6). The above results demonstrated that self-repair of the meniscal defects of both longitudinal and radial types was very limited and that the energy density of 100J/cm$^2$ stimulated better healing of meniscal defects.

Comparison of fibrochondrocytic proliferation in the avascular zones at 4 mm from the meniscal defect margin was made among groups I, II, and III (Fig. 7). The most significant proliferation with some dividing fibrochondrocytes was found in group II (Fig. 7C). Moderate cell multiplication was observed in group I (Fig. 7B). No significant cell proliferation was noted in the unlased group III (Fig. 7A).

### SEM Observations

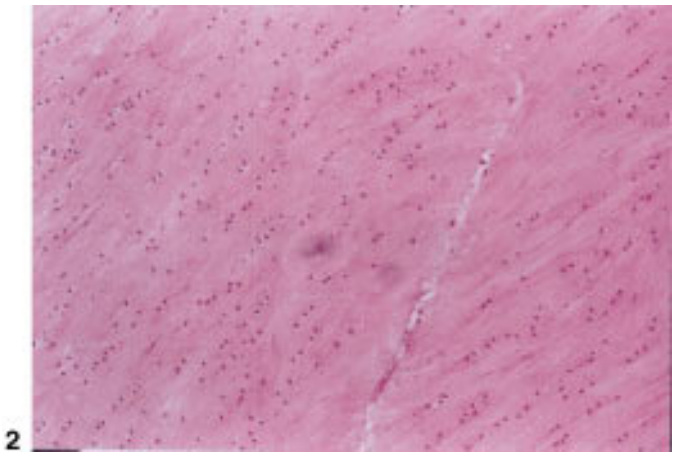
When examined under SEM, surface structures of meniscal defects were easily distinguished between the defect and its adjacent normal fibrocartilage. Complete repair of the longitudinal defect was observed in group II irradiated with 100 J. The full thickness defects in this group were covered with irregularly oriented collagen bundles of regenerated fibrocartilage on the surface of the healing defects, with a gap of only 15 μm (Fig. 8). The longitudinal meniscal defect irradiated with 50 J was partially repaired by moderate regenerated collagen fibers at the defect margins. In comparison, the control meniscal defect of longitudinal tear type showed only traces of regenerated collagen fibers with a gap of 50 μm in the middle (Figure 9).

Radial meniscal defects irradiated with 50 J at the concave edge showed signs of healing with moderate synthesis of collagen fibers (Fig. 10). However, no sign of healing was observed at the radial defect margins in the control group (Fig. 11).

## DISCUSSION

The results of this study indicate that healing of meniscal defect in an organ culture system can be promoted by low power $CO_2$ laser irradiation, when an optimal energy density of laser irradiation is used to stimulate fibrochondrocytic proliferation. The $CO_2$ laser energy levels selected for this study were based on our previous experience [16] on the beneficial effect of laser in tissue healing and the thermal harmful effects on meniscal cartilage. The power densities used in this experiment were much lower than reported that can cause tissue welding effect [17].

The effect of laser on cartilage repair depends on the type of laser, the energy density used, the mode of delivery, and type of tissue studied. Our results indicate that the meniscal healing potential of the avascular longitudinal defect surpassed that of the radial defect. In addition, the energy density of 100 J/cm$^2$ of $CO_2$ laser irradiation on meniscus enhanced the healing process. Abundant fibrochondrocytes were observed surrounding the longitudinal meniscal defects, which were nearly repaired and were filled with



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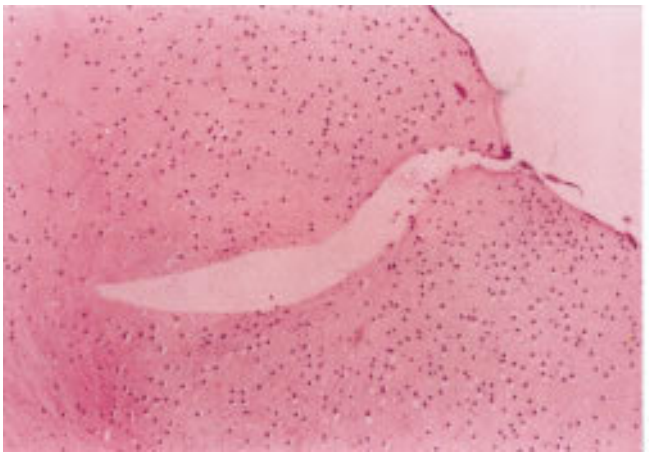

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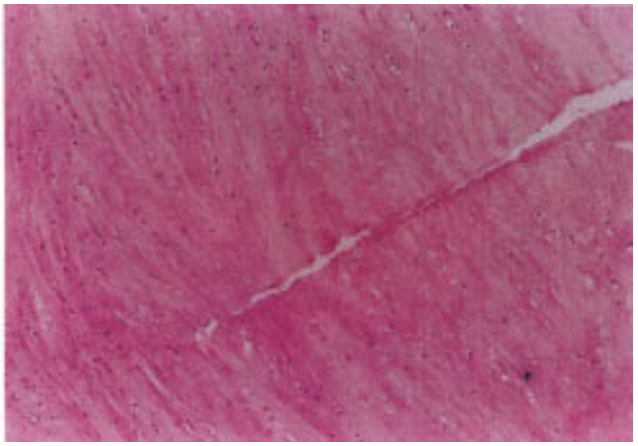

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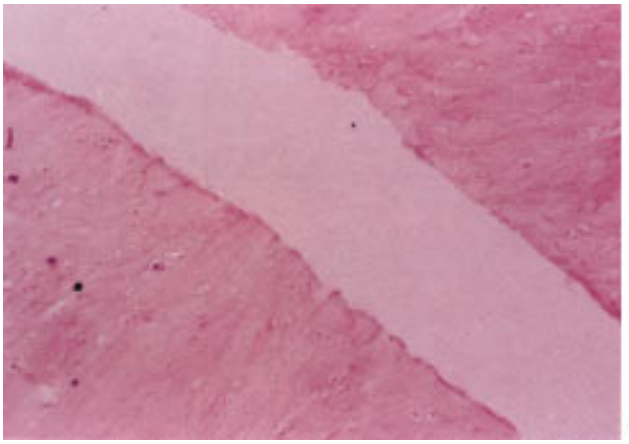

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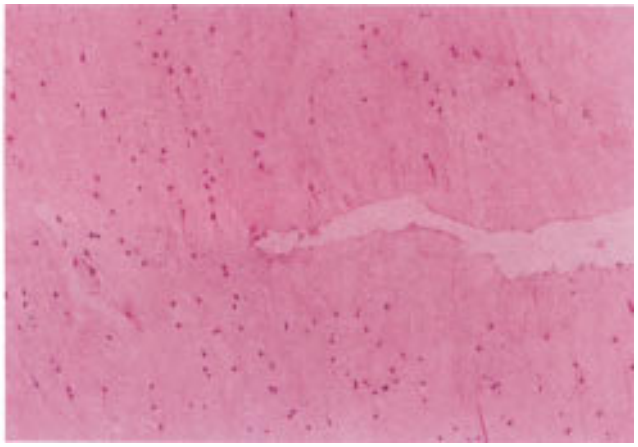

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Fig. 2. Photomicrograph of a longitudinal meniscal defect after irradiated with 100 J/cm$^2$ of $CO_2$ laser. The defect was filled with abundant matrix and fibrochondrocytes at the end of 8-week organ culture (LM, H & E, 100 ×).

Fig. 3. Photomicrograph of a longitudinal meniscal defect after irradiated with 50 J/cm$^2$ of $CO_2$ laser. Partial filling of the defect with regenerated fibrocartilage was observed at the end of 8-week organ culture (LM, H & E, 100 ×).

Fig. 4. Photomicrograph of a meniscal defect with a single longitudinal cut. No obvious repair on the unlased meniscus was observed after an organ culture of 8 weeks (LM, H & E, 100 ×).

Fig. 5. Photomicrograph of a radial meniscal defect after irradiated with 100 J/cm$^2$ of $CO_2$ laser. Fibrochondrocytic proliferation along the radial defect was observed at the end of 8-week organ culture (LM, H & E, 100 ×).

Fig. 6. Photomicrograph of a meniscal defect with radial type without any laser irradiation. No fibrochondrocytic proliferation was observed at the end of 8-week organ culture (LM, H & E, 100 ×).

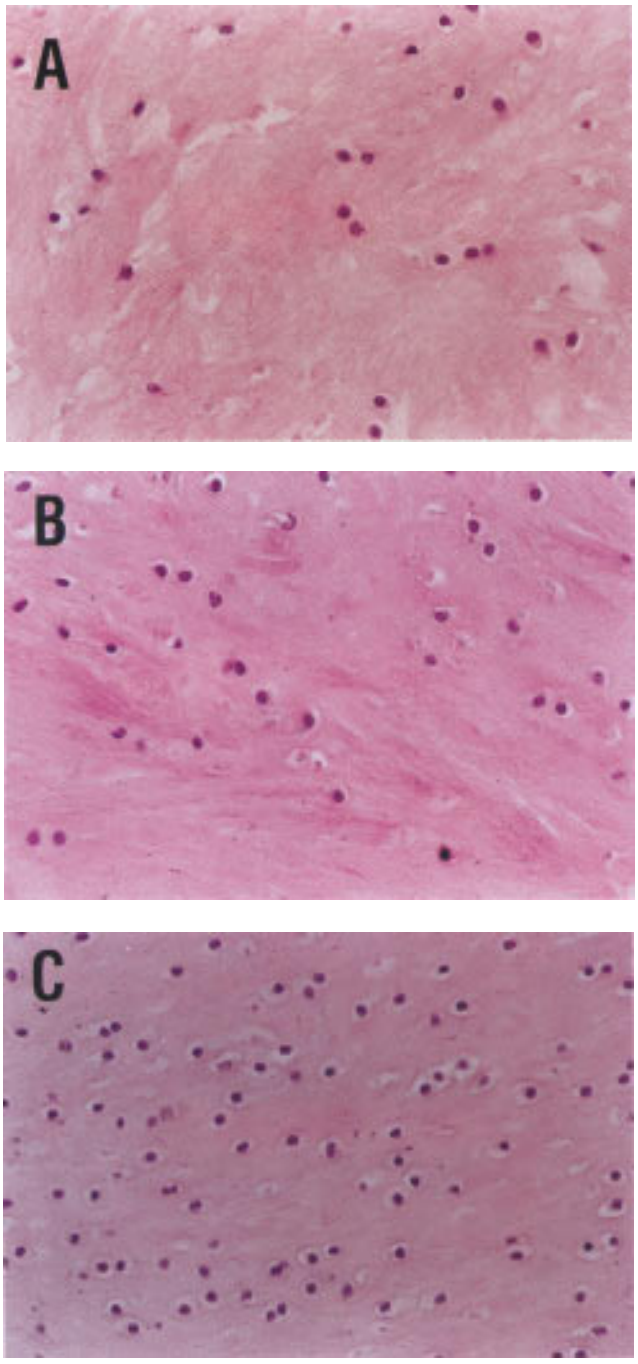


Fig. 7. Photomicrographs of fibrochondrocytes adjacent to longitudinal meniscal defects in groups I, II, and III at the end of 8-week organ culture (LM, H & E, 400 ×). **A.** No sign of fibrochondrocyte multiplication in unlased group III. **B.** Moderate increase of fibrochondrocytes in group I (irradiation with 50 J/cm$^2$ of $CO_2$ laser). **C.** Significant fibrochondrocyte proliferation in group II (irradiation with 100 J/cm$^2$ of $CO_2$ laser).

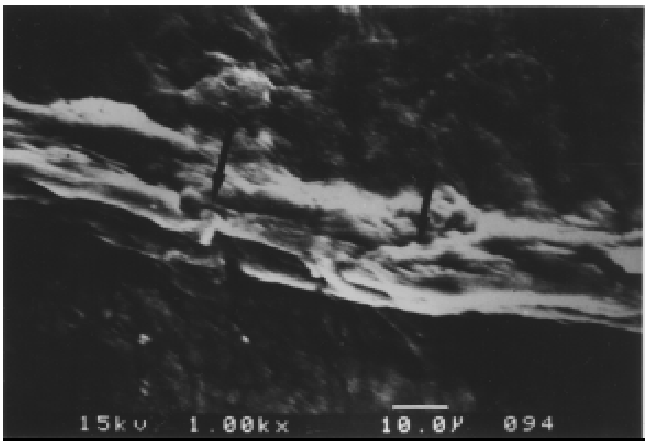


Fig. 8. Scanning electron micrograph of a longitudinal meniscal defect after irradiated with 100 J/cm$^2$ of $CO_2$ laser and cultured for 8 weeks. Arrow indicates regenerated fibrocartilage filled in the longitudinal cut (SEM, 1000 ×).

regenerated collagen fibers. Schultz et al. [11] reported that low power neodymium:yttrium-aluminum-garnet (Nd:YAG) laser energy can stimulate chondrocyte proliferation in articular cartilage of guinea pig knee joint. Conflicting in vivo data were reported on the ability of Nd:YAG laser energy to stimulate the healing of partial thickness cartilage defects [11,18]. The differences in laser intensity, the mode of laser delivery, and animal models used may account for the inconsistent results. Regarding the effect of laser on meniscal tears, Forman et al. [19] demonstrated that continuous wave argon ion laser can facilitate fibrin clot bonding of meniscal tears, resulting in an increased tensile strength of the me-

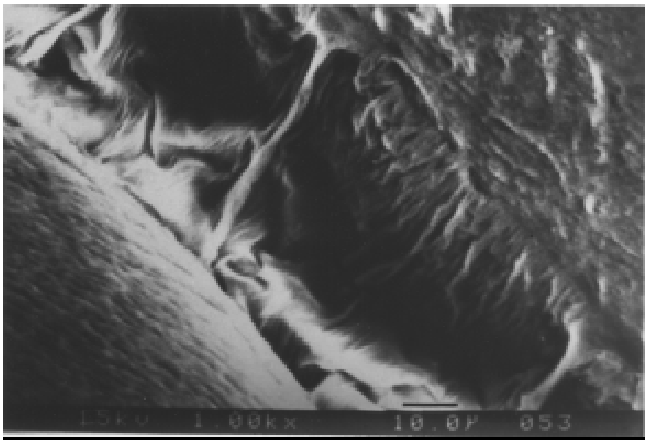


Fig. 9. Scanning electron micrograph of a longitudinal meniscal defect of unlased control group III. A gap of ~50μm was observed at the defect. Only partial fibrocartilage regeneration was observed in the middle layer (SEM, 1000 ×).


Fig. 10. Scanning electron micrograph of a radial meniscal defect after irradiated with 50 J/cm$^2$ of $CO_2$ laser and cultured for 8 weeks. Collagen regeneration along the radial defect was observed indicating ongoing healing process (SEM, 500 ×).

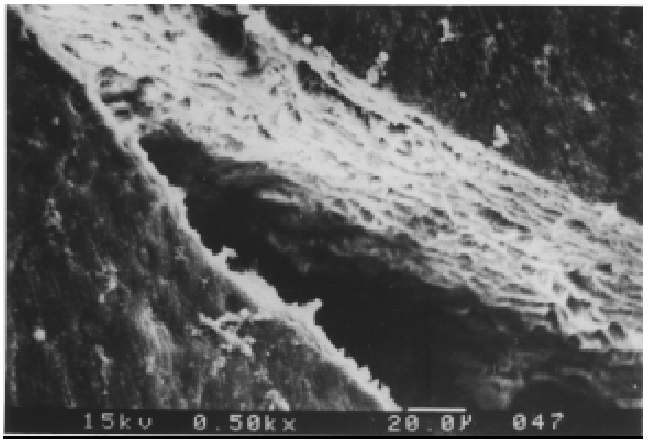


Fig. 11. Scanning electron micrograph of a radial meniscal defect of unlased control group III after 8-week organ culture. No obvious fibrocartilage regeneration was observed (SEM, 500 ×).

niscus 40 times that of the nonirradiated fibrin clot bonded meniscus.

Our results of increased fibrochondrocytic proliferation after $CO_2$ laser irradiation in meniscal organ culture were in accordance with those of in vitro studies of Nd:YAG laser irradiation on cartilage metabolism [12]. Nd:YAG laser irradiation of energies from 30 J to 100 J, delivered at 1.5 W power output, is stimulatory to cartilage metabolism by stimulating DNA synthesis of isolated chondrocytes. In addition, proteoglycan recovery is significantly enhanced in explants of human osteoarthritic cartilage when irradiation by 60 J of Nd:YAG laser.

In our study, increased fibrochondrocytic proliferation in the cultured rabbit meniscus was observed by light microscopy and elevated collagen fiber regeneration was demonstrated by scanning electron microscopy. Both fibrochondrocytic proliferation and collagen fiber regeneration are essential for meniscal healing. Mechanisms of these events have been investigated by researchers in various animal experiments with laser irradiation [20,21]. Some experimental data demonstrated that laser irradiation stimulates collagen synthesis, which is necessary for tissue repair [20]. Tang et al. [21] also demonstrated several favorable effects of low power $CO_2$ laser irradiation on the experimental bone fracture healing in rabbits. Events such as absorption of the hematoma, debridement of the necrotic tissues, activation of fibroblasts and chondrocytes, and increase of capillary formation can all be enhanced by low-power $CO_2$ laser irradiation. Spivak et al. [22] applied a noncontact, continuous wave Nd:YAG laser beam to full thickness adult articular cartilage explants maintained in organ culture. They found that Nd:YAG laser energies of 51-127 J/cm$^2$ can stimulate matrix synthesis at 6-7 days following laser exposure and the dose-dependent effects subsided by 12-14 days. Meniscal healing stimulated by low power $CO_2$ laser system was demonstrated in our study. Further investigation is necessary to elucidate whether the mechanism was due to biostimulation on fibrochondrocytes, chemotactic effect produced from thermal degradation of extracellular matrix, or combination of both factors attributed by laser energy.

Cartilage is one of those human tissues that usually does not repair itself clinically after major injury. The enhancement of healing of cartilage by simple and noninvasive laser therapy may have important future clinical implications and is worthy of further investigations.

## ACKNOWLEDGMENTS

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